Parasites as biological tags of divergence of black-striped pipefish, *Syngnathus abaster* (Actinopterygii: Syngnathiformes: Syngnathidae), populations in their natural and acquired range

Volodymyr YURYSYHNETS¹, Yuriy KVACH²,³, Iryna SYNIAVSKA⁴, Oleksandra SHEVCHENKO⁵, Yuliia KUTSOKON⁴

1 Institute of Hydrobiology, National Academy of Science of Ukraine, Kyiv, Ukraine
2 Institute of Marine Biology, National Academy of Science of Ukraine, Odesa, Ukraine
3 Odesa I. I. Mechnikov National University, Odesa, Ukraine
4 Schmalhausen Institute of Zoology, National Academy of Science of Ukraine, Kyiv, Ukraine
5 Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

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Corresponding author: Yuriy Kvach (yuruy.kvach@gmail.com)

**Abstract**

The presently reported study was intended to describe the current range of an Atlanto–Mediterranean fish, the black-striped pipefish, *Syngnathus abaster* Risso, 1827, in Ukrainian waters and to analyze biological tags (size parameters and parasites) of its different populations. The parasitological survey was carried at five different localities, including one marine site, two deltaic zones, and two localities in the middle Dnipro basin. The study provides comprehensive new data on parasites of the black-striped pipefish in Ukraine, with supporting data on its newly acquired freshwater range. A total of 21 parasite species (taxa) were revealed. Several parasite species were recorded for the first time on this host, i.e., *Trichodinella epizootica* (Raabe, 1950); *Trypanosoma sp.*; *Bothriocephalus scorpii* (Müller, 1776); *Progrillotia dasyatidis* Beveridge, Neifar et Euzet, 2004; *Ophiotaenia europaea* Odening, 1963; *Cryptocotyle jejuna* (Nicoll, 1907); *Metorchis xanthosomus* (Creplin, 1846); *Tylodelphys clavata* (von Nordmann, 1832); *Holostephanus luehei* Szidat, 1936; *Contracaecum rudolphii* Hartwich, 1964; *Mothocya epimerica* Costa in Hope, 1851; and Unionidae gen. sp. Formation of the species’ parasite component community depends entirely on environmental factors, with local parasite community features forming due to 1) presence of “marine” unicellular parasite species (ciliates) in marine localities (10‰–17‰ salinity) only, the community forming as a refraction of relative stenohalinity (*Trichodina rectuncinata* Raabe, 1958), findings of “marine” ciliate species in freshwater locations representing examples of successful osmoconformation (*Trichodina partiodes* Lom, 1962); or 2) presence of multicellular parasites in localities with abiotic/biotic conditions that allow completion of complex life cycles, such as those of trematodes (freshwater/marine mollusks as obligate first hosts) or cestodes (freshwater/marine invertebrates as intermediate hosts or marine/freshwater vertebrates as definitive hosts).

**Keywords**

brackish water, freshwater, Mediterranean species, neolimnetics, range extension, Ukraine
Introduction

Species range extension is a natural process stretched over time and it ultimately contributes to the formation of biodiversity (Alexandrov et al. 2007; Polačík et al. 2008). However, over recent decades, human activity has caused natural range borders to change significantly, spreading many species, including fishes (Bij de Vaate et al. 2002; García-Berthou et al. 2005; Hirsch et al. 2016). As a result, non-native (or alien) species are now having adverse effects (e.g., Ponto–Caspian gobïids in the Rhine River basin and the North-American Great Lakes, rainbow trout in European mountain locations, etc.) in many recipient ecosystems and, as such, they represent one of the biggest challenges for global biodiversity today (Leppäkoski et al. 2002; Hirsch et al. 2016).

Ukraine is located at a major crossing of transport corridors (land, freshwater, and marine), representing important routes for the spread of alien aquatic species (Alexandrov et al. 2007; Semenchenko et al. 2016). At least two crucial aquatic biological invasion routes pass through Ukraine’s aquatic ecosystems, the so-called Southern and Central Corridors (Panov et al. 2009), and many Ponto–Caspian and Mediterranean species have now increased their ranges along these corridors (Tutman et al. 2012; Semenchenko et al. 2016; Marenkov 2018; Dobrzycka-Krahel et al. 2023). One such group is the “neolimnetics”, which have a marine/brackish water origin but have spread into freshwater habitats in Ukraine and other European countries. These include species such as the Ponto–Caspian gobïids, the Ukrainian stickleback, *Pungitius platygaster* (Kessler, 1859); the black-striped pipefish, *Syngnathus abaster* Risso, 1827; and the big-scale sand smelt, *Atherina boyeri* Risso, 1810 (see Kvach and Kutsokon 2017).

The black-striped pipefish is an Atlanto–Mediterranean fish species with a natural range from the southern Gulf of Biscay in the north to Gibraltar in the south, the Mediterranean and Black Seas, and the estuarine zones of their rivers (Dawson 1986). It has a maximum body length of 21.5 cm (more commonly 16.0 cm) and a maximum weight of 3.0 g (age unknown). It lives along the coastal zone, mainly in shallow waters, and is tolerant of waters with different salinities (e.g., it is found along the coast of Crimea and in the Dnipro River near Kyiv), though it is far more common and numerous in estuaries of their rivers (Dawson 1986). It has a maximum body length of 21.5 cm (more commonly 16.0 cm) and a maximum weight of 3.0 g (age unknown). It lives along the coastal zone, mainly in shallow waters, and is tolerant of waters with different salinities (e.g., it is found along the coast of Crimea and in the Dnipro River near Kyiv), though it is far more common and numerous in estuaries of their rivers (Dawson 1986). It has a maximum body length of 21.5 cm (more commonly 16.0 cm) and a maximum weight of 3.0 g (age unknown). It lives along the coastal zone, mainly in shallow waters, and is tolerant of waters with different salinities (e.g., it is found along the coast of Crimea and in the Dnipro River near Kyiv), though it is far more common and numerous in estuaries of their rivers (Dawson 1986).

The fish were sampled using the 10 × 1 m dipnet and a 1 × 0.5 m diameter hand net (0.5 cm mesh). In total, 107 fish were sampled from five different localities during the warm seasons of 2020–2021 (Table 1; Fig. 1). These included one marine locality (Gulf of Odesa), two deltaic zones (Danube and Dniester deltas), and two localities in the middle Dniipro basin (Lake Vyzaky and the Stuhna River). In the Danube Delta, fish were sampled from three different sites (Fig. 1), considered the same locality.

![Table 1. Number (n) and standard length (SL) of *Syngnathus abaster* from different localities in Ukraine.](image)

<table>
<thead>
<tr>
<th>Locality</th>
<th>Geographic coordinates</th>
<th>n</th>
<th>Standard length Mean ± SD</th>
<th>Min–max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Sea (Gulf of Odesa)</td>
<td>46.443020; 30.772734</td>
<td>37</td>
<td>114.9 ± 22.8</td>
<td>65–203</td>
</tr>
<tr>
<td>Danube Delta</td>
<td>45.408702; 29.583393</td>
<td>20</td>
<td>107.5 ± 18.3</td>
<td>78–145</td>
</tr>
<tr>
<td>Dniester Delta</td>
<td>46.466634; 30.197059</td>
<td>17</td>
<td>104.1 ± 29.0</td>
<td>58–166</td>
</tr>
<tr>
<td>Dniipro basin</td>
<td>Lake Vyzaky</td>
<td>15</td>
<td>111.5 ± 16.2</td>
<td>79–140</td>
</tr>
<tr>
<td></td>
<td>Stuhna River</td>
<td>16</td>
<td>102.9 ± 24.0</td>
<td>51–143</td>
</tr>
</tbody>
</table>

Parasites are commonly used as biological tags of their host populations (Moser 1991; Mackenzie 2002). Fish parasite communities can be used to discriminate fish stocks and populations and, as such, a process known as “biological tagging” (Catalano et al. 2014; Poulin and Kamilya 2015; Kvach et al. 2017; Kutsokon et al. 2022) and the same biological tag can be used as a marker to clarify the possible origin of invasive populations (Hohenadler et al. 2018a, 2018b; Ondračková et al. 2012b; 2019; 2021). A total of 18 parasite species have been recorded for populations of the Black Sea basin black-striped pipefish (*Gaevskaya et al. 1975*). However, data for parasites in its acquired range are primarily related to the Danubian population in Bulgaria, where nine species were recorded (Ondračková et al. 2012a), and the Dniipro population in Ukraine, where just two ciliate species were recorded (*Trichodina partiidisc Lom, 1962* and *Trichodina acuta Lom, 1961*; see Yurishnets 2010). Consequently, very little is known about parasites acquired in the species’ non-native freshwater habitats.

Therefore, the presently reported study aimed to describe the current range of black-striped pipefish in Ukrainian waters and obtain biological tag data (parasites) for the populations.
ty for further analysis. The salinity of the Gulf of Odesa is 10‰–17‰ depending on the season (Zaitsev 1992). Once caught, the fish were transported alive to the laboratory in aerated cans filled with water from the sampling site, where they were placed in aerated aquaria containing water from the place of capture for no more than two days (Kvach et al. 2016) before being examined for parasites.

Findings of black-striped pipefish from other sites were registered (without removing the fish) and used to evaluate the range of the species (Fig. 1). In addition, a literature search was undertaken (Movchan 2012; Movchan and Roman 2014; Demchenko 2017; Kutsokon and Kvach 2021; Kutsokon and Roman 2021; Kutsokon et al. 2021a, 2021b) to obtain the latest information on the species’ present-day range.

Prior to dissection, the standard length (SL, mm) and sex was determined for each fish (Table 1). After humanely sacrificing the fish, smears of gill and fins mucosa were taken, dried, and stained according to Klein (1958) to identify ciliates. Likewise, blood smears were dried and stained in hematoxylin (Giemsa 1904) to identify other unicellular organisms. Fresh smears from muscle and the gall and uterine bladders were examined for myxozoans and any living spores mounted onto gelatin gel as semi-permanent preparations for further identification. Monogeneans were placed onto glass slides and mounted in glycerin-ammonium-picate for morphological study (Malmberg 1957). Cestodes, digeneans, and nematodes were fixed with hot 4% formalin (Cribb and Bray 2010), and glochidia and crustaceans in cold 4% formalin. Acanthocephalans and nematodes were mounted in glycerin as temporary slides for further species identification, while cestodes and digeneans were stained in iron acetocarmine and mounted onto Canada balsam slides (Georgiev et al. 1986). All parasites were identified to the lowest possible taxa, with parasite taxonomy presented following the World Register of Marine Species (WoRMS 2022). Parasitological terminology and principal indices, such as prevalence, intensity, mean intensity, and abundance, were used in accordance with Bush et al. (1997):

**Prevalence (P)**

\[ P = \frac{n}{N} \times 100\% \]

where \( n \) is the number of infected fish and \( N \) is the number of fish examined.

**Intensity** (of infection) \((I)\) is the number of individuals of a particular parasite species in a single infected host (individual fish) usually presented as the intensity range \((I_R)\) (minimum–maximum).

**Mean intensity** (of infection) \((I_M)\)

\[ I_M = \frac{N_{TP}}{N_{inf}} \]

where \( N_{TP} \) is the total number of individuals of a particular parasite species found in all fish examined and \( N_{inf} \) is the number of infected fish.

**Abundance (A)**

\[ A = \frac{N_{T}}{N} \]

where \( N_{T} \) is the total number of individuals of a particular parasite species found in all fish examined and \( N \) is the number of fish examined.

For microparasites (unicellular and myxozoans), only the prevalence was calculated, with the intensity of infection evaluated by the presence of microparasites in the microscope’s field of view (Mierzejewska et al. 2012) as:

- Sporadic (S), from 1 to < 10 individuals in the material examined;
- Not numerous (NN), < 10 individuals in < 10% of field of view;
- Numerous (N), up to 20 individuals in > 50% of field of view;
- Very numerous (VN), > 20 individuals in > 50% of field of view;
- Mass (M), dozens of individuals in each field of view.
The Czekanowski–Sørensen index (CSI) was calculated (Sørensen 1948) to analyze differences in parasite fauna at different localities, with differences considered high in cases where index parameters were < 50%.

Statistical differences in length were evaluated using t-tests and F-tests for comparing two samples in Statistica for Windows 10 (StatSoft). Standard deviation values (SD) were calculated for mean parameters in each case. Visualization of fish size data was carried out in PAST v.4.03 (Paleontological Statistics Software system), using the search statistics methods (box and whisker), comparative analysis, and cluster analysis (Hammer et al. 2001). Discriminant analysis was then performed to evaluate differences in the respective parasite communities.

Results

We observed no significant difference in the length of black-striped pipefish from marine (Gulf of Odesa), estuarine (Danube and Dniester deltas), or freshwater (Lake Vyazky and Stuhna River) localities (Fig. 2).

![Figure 2. Box (median, 50% sample size) and whisker (min, max) plot for size (SL, mm) of Syngnathus abaster populations from different localities in Ukraine.](image)

Based on published literature and databases, we consider the modern range of black-striped pipefish to include both brackish and freshwaters of the Black Sea basin (Fig. 1). In rivers where the populations have been established since the middle of the 20th century, the species is still to be found in the lower reaches in the presence of the lower first-order tributaries of the Dniipro, regardless of size (i.e., medium rivers such as the Ros and Desna, and small rivers such as the Leglych and Mokra Moskovka). Despite much long-term research in the area, the black-striped pipefish has not been found in upper-flow tributaries to date. While the highest recorded finding was in the middle flow of the Ros River in 1923 (Beling 1923), all later records of the species have been from the lower reaches of the river (Kutsokon 2010). The species is also found in the lower reaches of the Dniester, Danube (along the entire stretch within the borders of Ukraine), Tyligul, Southern Buh, and rivers of the Crimean and Northern Azov coasts (Demchenko 2017; Kutsokon and Kvak 2021; Kutsokon and Roman 2021; Kutsokon et al. 2021b).

The parasite fauna of Ukrainian black-striped pipefish consisted of 21 parasite species (taxa), including five unicellular taxa, four cestodes, nine digeneans, one nematode, one isopod, and unidentified glochidia: Trichodina spp., Trichodina partidisci Lom, 1962, Trichodina rectuncinata Raabe, 1958, Trichodinella epizoitica (Raabe, 1950) [CILIOPHORA]; Trypanosoma sp. [KINETOPLASTEA]; Proteocephalus sp., Bothriocephalus scorpii (Müller, 1776), Progrillotia dasyatidis Beveridge, Neifar et Euzet, 2004, Opisthoetaenia europea Odening, 1963 [CESTODA]; Orientocreadium pseudobagri Yamaguti, 1934, Nicolla skrjabini (Iwanitzky, 1928), Cryptocotyle concava (Creplin, 1825), Cryptocotyle jejuna (Nicoll, 1907), Timoniella imbutiformis (Molin, 1859), Metorchis xanthosomus (Creplin, 1846), Diplodostomum spp., Tylodelphys clavata (von Nordmann, 1832), Holostephanus luehei Szidat, 1936 [DIGENEA]; Contracaecum rudolphi Hartwich, 1964 [NEMATODA]; Mothocephus epimerica Costa in Hope, 1851 [CRUSTACEA]; Unionidae gen. sp. [BIVALVIA] (Table 2).

Parasite richness varied from 2–3 species in the Dnipro basin and up to eight species in the Black Sea. The majority of parasites were represented by larval stages, with just two digeneans—Orientocreadium pseudobagri, Nicolla skrjabini—and an isopod Mothocephus epimerica found as adults.

The most numerous species were ciliates of Trichodina spp., with the prevalence varying from 5.9% to 100% and intensity of infection of up to several thousand cells (Table 2). Among the ciliates, we identified at least three species, most represented by Trichodina partidisci, found in both freshwater (except the isolated Lake Vyazky) and marine/brackish sites. This is a small-sized species (23.7–31.5 µm; 27.6 ± 2.9), with a denticula ring diameter of 10.4–16.1 µm (13.5 ± 2.0) and a denticula number of 18–24 (22). It can be recognized by the broadly rounded distal surface of its denticula blade and the (usually) few, irregular unstained granules in the central part of its adhesive disc (Fig. 3). Trichodina rectuncinata, a species common on marine fish, was only found in the Gulf of Odesa. Again, this is a small-sized species (23.0–30.5 µm, 28.8 ± 1.7) with a denticula ring diameter of 12.3–15.2 µm (13.6 ± 1.2) and a denticula number of 22–25 (24), recognized by a triangular blade with a cavity in the center (Fig. 3). Trichodinella epizoitica, a small mobilid typical of freshwaters, was registered in just one fish from the Danube Delta.

Ukrainian pipefish populations differed with the abundance values for Nicolla skrjabini and Diplodostomum spp.
Table 2. Parasite communities of *Syngnathus abaster* from various localities in Ukraine (as determined in the presently reported study).

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Developmental stage</th>
<th>Location on host</th>
<th>Index</th>
<th>Black Sea (Gulf of Odesa)</th>
<th>Danube Delta</th>
<th>Dniester Delta</th>
<th>Dnipro basin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichodina</em> spp.</td>
<td>trophont</td>
<td>gills, skin, fins</td>
<td>P [%]</td>
<td>64.1</td>
<td>68.2</td>
<td>5.9</td>
<td>22.2</td>
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<tr>
<td><em>Trichodina rectuncinata</em></td>
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<tr>
<td><em>Trichodinella</em> epizootica</td>
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<tr>
<td><em>Trypanosoma</em> sp.</td>
<td>plerocercoid</td>
<td>blood</td>
<td>P [%]</td>
<td>4.5</td>
<td>1.0</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>proteocephalus</td>
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<tr>
<td><em>Proteocephalus</em> sp.</td>
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<td><em>Proglottis dasyatidis</em></td>
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<td><em>Proglottis</em></td>
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<td><em>Bothriocephalus scorpii</em></td>
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<td><em>Ophiotaenia</em></td>
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<td><em>O/re</em></td>
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<tr>
<td><em>Nicolla skrjabini</em></td>
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<td><em>Cryptocotyle concava</em></td>
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<td><em>Cryptocotyle jejuna</em></td>
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<tr>
<td><em>Timoniella imbatiformis</em></td>
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<tr>
<td><em>Metorchis santhosomus</em></td>
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<tr>
<td><em>Diplostomum</em> spp.</td>
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<tr>
<td><em>Tylodelphys clavata</em></td>
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</tr>
<tr>
<td><em>Holostephanus luehei</em></td>
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</tr>
</tbody>
</table>

**Index:** P [%], I, R, S–M, NN, VN, A

**Location on host:** Lake Vyazky, Stuhna River
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(Table 3), with a comparative analysis of parasite communities using the Czekanowski–Sørensen index (CSI) distinguished marine populations from freshwater/brackish populations (Fig. 4). CSI, Mahalanobis distance, and the Fischer criterium all showed significant differences between the Gulf of Odesa and the Danube and Dniester deltas (Table 4), with CSI parameters showing the largest differences between all localities.

**Discussion**

This study provides comprehensive new data on the parasites of black-striped pipefish in Ukraine, along with supporting data on its acquired range in Ukrainian freshwaters. Our new data confirm that the species is now found in the coastal zones of the Black Sea and the Sea of Azov and the deltaic zones of rivers and reservoirs of...
However, the only new findings we recorded were inside the species’ established range in the Lower Dnipro basin, suggesting that its Ukrainian range has probably now stabilized since it started spreading in the middle of the 20th century.

Several parasite species were registered on this host for the first time, i.e., *Trichodinella epizootica*, *Trypanosoma sp.*, *Bothriocephalus scorpii*, *Progrillotia dasyatidis*, *Ophiothela europaea*, *Proteocephalus sp.*, *Nicola skrjabini*, *Orientocreadium pseudobagri*, *Cryptocotyle concavum*, *Cryptocotyle jejuna*, *Metorchis xanthosomus*, *Tylodelphys clavata*, *Holostephanus luehei*, *Nicolla skrjabini*, *Orientocreadium pseudobagri*, *Cryptocotyle concavum*, *Cryptocotyle jejuna*, *Timoniella imbutiformis*, *Trichodina partidisci*, *Trichodina rectuncinta*, *Proteocephalus sp.*, *Progrillotia dasyatidis*, *Ophiotaenia europaea*, *Cryptocotyle jejuna*, *Metorchis xanthosomus*, *Contracaecum rudolphii*, *Mothocya epimerica*; and *Unionidae gen. sp.* (Table 2). In addition, we confirmed the presence of several species previously recorded on the black-striped pipefish, including several ciliate species. While these are common on a wide spectrum of freshwater and brackish water hosts (Kostenko 1981; Grupcheva et al. 1989; Yurishinets 2010), no specific ciliates were previously known for black-striped pipefish. The most common species found was *Trichodina partidisci*, a parasite of mugilid fish in the Black Sea (Lom 1962) that has a wide spectrum of hosts (Grupcheva et al. 1989; Lom and Dyková 1992). In Ukrainian freshwaters, it is known from pipefish in the middle Dnieper basin (Yurishinets 2010). A second species, *Trichodina rectuncinta*, a widespread marine fish parasite, has previously been documented in the Dnipro basin (Movchan 2011; Snigirov et al. 2020).

### Table 3. Discriminant function analysis of parasite communities of *Syngnathus abaster* from different localities in Ukraine.

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Wilks’ Lambda</th>
<th>Partial Lambda</th>
<th>F-remove (4,89)</th>
<th>P-value</th>
<th>Toler.</th>
<th>1-Toler. (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichodina</em> spp.</td>
<td>0.42</td>
<td>0.91</td>
<td>2.08</td>
<td>0.09</td>
<td>0.59</td>
<td>0.41</td>
</tr>
<tr>
<td><em>Trypanosoma</em> sp.</td>
<td>0.41</td>
<td>0.93</td>
<td>1.60</td>
<td>0.18</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Bothriocephalus</em> scorpii</td>
<td>0.41</td>
<td>0.95</td>
<td>1.15</td>
<td>0.34</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Ophiothela</em> europaea</td>
<td>0.41</td>
<td>0.93</td>
<td>1.60</td>
<td>0.18</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Proteocephalus</em> sp.</td>
<td>0.40</td>
<td>0.95</td>
<td>1.06</td>
<td>0.38</td>
<td>0.99</td>
<td>0.01</td>
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<tr>
<td><em>Progrillotia</em> dasyatidis</td>
<td>0.39</td>
<td>1.00</td>
<td>0.02</td>
<td>1.00</td>
<td>0.54</td>
<td>0.46</td>
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<td><em>Nicola</em> skrjabini</td>
<td>0.44</td>
<td>0.89</td>
<td>2.86</td>
<td>0.03</td>
<td>0.97</td>
<td>0.03</td>
</tr>
<tr>
<td><em>Orientocreadium</em> pseudobagri</td>
<td>0.42</td>
<td>0.91</td>
<td>2.16</td>
<td>0.08</td>
<td>0.96</td>
<td>0.94</td>
</tr>
<tr>
<td><em>Cryptocotyle</em> concavum</td>
<td>0.39</td>
<td>1.00</td>
<td>0.10</td>
<td>0.98</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td><em>Cryptocotyle</em> jejuna</td>
<td>0.39</td>
<td>1.00</td>
<td>0.03</td>
<td>1.00</td>
<td>0.03</td>
<td>0.97</td>
</tr>
<tr>
<td><em>Timoniella</em> imbutiformis</td>
<td>0.39</td>
<td>0.98</td>
<td>0.45</td>
<td>0.77</td>
<td>0.99</td>
<td>0.01</td>
</tr>
<tr>
<td><em>Diplostomum</em> spp.</td>
<td>0.45</td>
<td>0.87</td>
<td>3.39</td>
<td>0.01</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Tylodelphys</em> clavata</td>
<td>0.41</td>
<td>0.93</td>
<td>1.60</td>
<td>0.18</td>
<td>0.98</td>
<td>0.02</td>
</tr>
<tr>
<td><em>Holostephanus</em> luehei</td>
<td>0.42</td>
<td>0.92</td>
<td>1.98</td>
<td>0.11</td>
<td>0.84</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Metorchis</em> xanthosomum</td>
<td>0.41</td>
<td>0.94</td>
<td>1.31</td>
<td>0.27</td>
<td>0.06</td>
<td>0.94</td>
</tr>
<tr>
<td><em>Contracaecum</em> rudolphii</td>
<td>0.39</td>
<td>0.99</td>
<td>0.28</td>
<td>0.89</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td><em>Mothocya</em> epimerica</td>
<td>0.39</td>
<td>1.00</td>
<td>0.01</td>
<td>1.00</td>
<td>0.25</td>
<td>0.75</td>
</tr>
<tr>
<td><em>Unionidae</em> gen. sp.</td>
<td>0.40</td>
<td>0.96</td>
<td>0.89</td>
<td>0.48</td>
<td>0.92</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Wilks’ Lambda: approx. 0.38655; $F(72,352) = 1.3380; P < 0.05$; **Bold** font dentotes significant differences.

### Table 4. Matrix of differences between parasite fauna/communities of pipefish from different localities.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Index</th>
<th>Black Sea (Gulf of Odesa)</th>
<th>Danube Delta</th>
<th>Dniester Delta</th>
<th>Lake Vyazky</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danube Delta</td>
<td>ICS</td>
<td>28.57</td>
<td>100.00</td>
<td>0.00</td>
<td>1.78</td>
</tr>
<tr>
<td>MD</td>
<td></td>
<td>2.72</td>
<td>4.21</td>
<td>4.27</td>
<td>4.21</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>1.78</td>
<td>2.33</td>
<td>1.91</td>
<td>1.91</td>
</tr>
<tr>
<td>Dniester Delta</td>
<td>ICS</td>
<td>13.33</td>
<td>30.77</td>
<td>100.00</td>
<td>2.33</td>
</tr>
<tr>
<td>MD</td>
<td></td>
<td>4.21</td>
<td>4.27</td>
<td>0.00</td>
<td>4.21</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>2.33</td>
<td>1.91</td>
<td>1.91</td>
<td>1.91</td>
</tr>
<tr>
<td>Lake Vyazky</td>
<td>ICS</td>
<td>0.00</td>
<td>0.00</td>
<td>22.22</td>
<td>0.00</td>
</tr>
<tr>
<td>MD</td>
<td></td>
<td>2.42</td>
<td>2.78</td>
<td>3.92</td>
<td>2.78</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>1.22</td>
<td>1.16</td>
<td>1.46</td>
<td>1.16</td>
</tr>
<tr>
<td>Stuhna River</td>
<td>ICS</td>
<td>18.18</td>
<td>22.22</td>
<td>40.00</td>
<td>40.00</td>
</tr>
<tr>
<td>MD</td>
<td></td>
<td>1.72</td>
<td>2.31</td>
<td>3.12</td>
<td>2.31</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.99</td>
<td>1.07</td>
<td>1.27</td>
<td>1.07</td>
</tr>
</tbody>
</table>

CSI = Czekanowski–Sørensen index, MD = squared Mahalanobis distances, F = Fischer criterium. Significant differences ($P < 0.05$) are marked in **bold**.

Figure 4. Dendrogram of similarity expressed by Czekanowski–Sørensen index for parasites component communities of *Syngnathus abaster* at different localities in Ukraine.
recorded in different areas of the Atlantic and Pacific oceans (Xu et al. 2001; Islas-Ortega et al. 2020; Öztürk and Güven 2022).

All four cestode species on black-striped pipefish were only registered at the pterocercoid stage. Two of the cestodes, Bothriocephalus scorpiei and Progillotilia dasyatis, were marine species reported in the Gulf of Odesa. Bothriocephalus scorpiei has been known as a parasite of turbots, Scophthalmus maximus (Linnaeus, 1758), scorpionfish (Scorpaenidae), mullet (Mugilidae), mackerel (Scombridae), and rays (Batoidea); Progillotilia dasyatis is a parasite of a stingray, Dasyatis pastinaca (Linnaeus, 1758) (see Beveridge et al. 2004; Kuchta et al. 2008). While Progillotilia dasyatis is common in fishes from the Gulf of Odesa (Kvach et al. 2022), Bothriocephalus scorpiei has only been registered in adjacent water bodies (Kvach 2010). 

Proteocephalus sp. (see Fig. 3b), recorded in pipefish from the Danube, is a common parasite of Danubian freshwater fishes (Bauer 1985) and has previously been reported in pipefish from the middle Danube (Ondráčková et al. 2012a). Finally, Ophiotocaenia europea (see Beveridge et al. 2004), is a parasite of grass snakes that uses fishes as its paratenic hosts (Sharpilo and Monchenko 1971). While it has previously been registered in fish from the Danube Delta (Kvach et al. 2020), this is the first time it has been registered in the Dniester River.

While the freshwater digeneans, Oriatocreadium pseudobagri and Nicolla skryjabini, were recorded as adults in the gut of pipefish from the Danube and Dniester deltas (Table 2; Fig. 3), other digeneans were only represented by metacercariae, with either marine fish as their definitive hosts (Timoniella imbutiformis) or fish-eating birds (Cryptocotyle concava, Cryptocotyle jejuna, Metorchis xanthosomus, Diplostomum sp., Tylodelphys clavata). Moreover, we have not recorded those metacercariae with a wide spectrum of hosts, e.g., Cyathocotylidae fam. spp., Echinococchus perfoliatus (Ratz, 1908), and Metagonimus sp., despite these having previously been reported in pipefish from the middle Danube basin (Ondráčková et al. 2012a).

In the Gulf of Odesa and the Danube Delta, sporadic cases of parasitism by Contracaecum rudolphii nematode larvae were noted, the adult worms being common parasites of pelicans, herons, mergansers, and cormorants (Sreedevi et al. 2017). Previous authors (e.g., Gaeviskaya et al. 1975) have also noted the presence of another species of this genus, Contracaecum microphalum (Rudolphi, 1809), along with larvae of Agamonema sp. nematode in the parasite fauna of marine pipefish.

The isopod Mothocya epimera, a parasite of the branchial and oral cavities of sand-smelts (Atherina spp.), was only noted at a marine location with few indications of invasion (Bruce 1986; Leonardos and Trillos 2004). The parasitic copepods Ergasilus lizae Kröyer, 1863 and Ergasilus ponticus Markевич, 1940, have also previously been reported from marine populations of the black-striped pipefish (Gaeviskaya et al. 1975).

Parasitism on the gills of unionid bivalve larvae (Unionidae) was noted for three of four freshwater localities, though with relatively low invasion rates (P = 5%–7%, I = 2–5 ind.).

The acanthocephalans Paracanthocephaloides incrassatus (Molin, 1858) (see Gaeviskaya et al. 1975) and Acanthocephaloides irregularis (Amin, Oğuz, Heckman, Tepe & Kvach, 2011) have both been recorded in pipefish populations from Black Sea localities, while the acquisition of Pomphorhynchus laevis (Zoega in Müller, 1776) has been confirmed from the middle Danube (Ondráčková et al. 2012a). We have not found, however, the above-mentioned parasite species during the presently reported study.

The black-striped pipefish is now widespread in Ukrainian bodies of water, particularly in coastal brackish and freshwaters of the Black Sea and the Sea of Azov basins. In Ukraine, it is found along all shores of the Black and Azov Seas and in the estuaries, near-estuary and estuarine zones of their rivers, from where it has entered reservoirs and rivers connected to the sea (below the Danube, Dniester, and Southern Buh). It is also found in all reservoirs along the Dnipro and the Siversky Donets River (Movchan 2011). Previous studies have confirmed differences in biotope preferences between marine pipefish, which prefer plant thickets, and those in freshwaters, which prefer muddy biotopes (Ondráčková et al. 2012a; middle Danube).

Ondráčková et al. (2012a) noted the absence of any parasites specific to this fish species, suggesting that the formation of the species’ parasite component communities depends entirely on the environmental factors affecting each population. In such cases, the local features of the parasite communities will depend on the following:

- The presence of “marine” species of unicellular parasites (ciliophores) in marine localities (10%–18% salinity) only as a refraction of relative stenohalinity (Trichodina rectuncinata), or findings of “marine” ciliate species in freshwater locations, as an example of successful osmocoonformation (Trichodina partidisci).
- The presence of multicellular parasites in localities with abiotic/biotic conditions that allow completion of complex life cycles, i.e., trematodes (freshwater/marine mollusks as obligate first hosts) or cestodes (freshwater/marine invertebrates as intermediate hosts or marine/freshwater vertebrates as definitive hosts).

Overall, the parasite fauna of neolimnetic black-striped pipefish exhibits two main parasite community formation strategies in their acquired ecosystems:

- Parasite release (very poor communities in freshwater).
- Acquisition of local parasite species, which have overcome the filters of encounter and adaptation (Combes 1995).

Our findings confirm that analyzing changes in the structure of neolimnetic fish parasitic communities that over-
come geographical and ecological barriers is a convenient model for establishing the patterns and features of hydrobiotic distribution beyond the boundaries of natural habitats.

Acknowledgments

This study was partly developed under the framework of the project “Development of scientific backgrounds of comprehensive monitoring and threats of distribution of invasive fish species by riverine systems and transitional waters of Ukraine (based on the parasite, population, and genetic markers)” (#2020.02.0171; National Research Foundation of Ukraine). We want to thank Mykola Prychepa and Yulia Kovalenko for kindly providing the sample from Lake Vyazky and for their help with basic laboratory processing. In addition, we thank Dr Kevin Roche for help with English proofreading.

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